ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Prevalence of *C. difficile* in patients with antibiotic associated diarrhea in a tertiary care hospital.

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Manuscript Info

Manuscript History:

Received: 12 April 2014
Final Accepted: 15 May 2014
Published Online: June 2014

Key words:
C. difficile, antibiotic associated diarrhea, AAD, *C. difficile* toxin A+B ELISA

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Abstract

Context: *C. difficile* is a major cause of hospital acquired diarrhea.

Aims: A prospective study was carried out to determine the prevalence of *C. difficile* in patients with antibiotic associated diarrhea (AAD) in a tertiary care hospital.

Methods and Material: 162 stool samples were examined over a two year period for toxin A and toxin B via *C. difficile* Toxin A+B Stool Antigen Microwell ELISA kit (IVD Research Inc.). Clinical and demographic data was collected from all patients.

Results: The prevalence of *C. difficile* associated diarrhea was found to be 4.32%. Highest number of C. *difficile* toxin positive cases was from stool samples of patients admitted in oncology/ hematology followed by internal medicine. Median time of occurrence of symptoms was 7 days after admittance to hospital. All the patients were on multiple antibiotics.

Conclusion: This present prospective study draws attention to the role of *C. difficile* as a cause of AAD in our hospital. Active and aggressive surveillance and sampling before putting patients empirically on anti- *C. difficile* treatment will determine the true prevalence of *C. difficile* in our hospital. Timely laboratory results of *C. difficile* testing can impact decisions regarding antibiotic therapy and infection control measures.

INTRODUCTION

Diarrhea is one of the most common complications associated with antibiotic therapy (Kelly andLaMont, 2008). Studies have revealed that *Clostridium difficile* is an important cause of antibiotic associated nosocomial diarrhea and is responsible for virtually all cases of pseudomembranous colitis (Freeman et al., 2010;Barbut et al., 2002). *C. difficile* is acquired exogenously, most frequently in the hospital or nursing home, and is carried in the stool of symptomatic and asymptomatic patients. The risk of *C. difficile* acquisition increases in proportion to the length of hospital stay. The bacterium is capable of producing both an enterotoxin, toxin A and a cytotoxin, toxin B (Alfa, 1998). In addition to *C. difficile* spore ingestion and toxin production, an alteration of the normal colonic flora,

typically caused by prior antibiotic use, is needed for clinical disease (Cardona and Rand , 2008; Aslam and Musher, 2006).

Although not all individuals who are colonized with toxigenic *C. difficile* strains exhibit bowel disease, early diagnosis is associated with better prognosis (Rüssmann et al., 2007).Therefore, rapid and accurate testing of stool samples is highly desirable. Most laboratories use a commercial enzyme immunoassay (EIA) to detect toxin A and /or toxin B, with the benefits of rapid turnaround time and ease of use (Shin et al., 2009). The exact prevalence of *C. difficile* as a cause of antibiotic associated diarrhea (AAD) in our hospital is not known. The aim of this study was to determine the prevalence of *C. difficile* in patients with AAD in our tertiary care hospital.

Subjects and Methods:

Specimen Collection: For this study, one hundred sixty two consecutive, non-duplicate fecal specimen submitted for *C. difficile* testing at Sher-i- Kashmir Institute of Medical Sciences over a two year period were included. Ethical clearance was obtained from Institute's ethical clearance committee. Indications of testing were either clinical suspicion of AAD or diarrhea occurring after 3 days post admission. Only diarrheal fecal specimen (i.e., those that adopted the contours of the vessel) were included in the study. Stool specimens were frozen at -20°C as per manufacturers' recommendations whenever fresh samples could not be tested within 48 hrs. Multiple cycles of freezing and thawing were avoided, because this procedure is known to have detrimental effect upon *C. difficile* cytotoxins. The test was performed in batches once a week.

Collection of patient data: Data were collected from all patients whose fecal samples were tested for *C. difficile* toxin. These data included demographics, admission details, lengths of hospital stay before diarrhea, and histories of non-antibiotic (antacids, anti peristaltics, cytotoxics, and gut stimulants) and antibiotic (aminoglycosides; narrow-, expanded-, and broad spectrum cephalosporins; co-trimoxazole; fluoroquinolones; glycopeptides; macrolides; metronidazole; and broad- and narrow-spectrum penicillins) drug administration and procedures undertaken (endoscopy, enema, feeding tube insertion, and surgery) within the 28 days before the onset of symptoms.

Assay of stool specimen for toxin A (tcdA) and toxin B (tcdB): Stool samples were examined for tcdA and tcdB via *C. difficile* Toxin A+B Stool Antigen Microwell ELISA kit (IVD Research Inc.). ELISA used in this study was a qualitative 96 well microplate assay for detection of toxin A and toxin B of *C. difficile*. Assay was carried out and interpreted as per the manufacturers' instructions. The test was performed from the portion of the stool homogenized with a wooden applicator stick after thawing the stored specimen at room temperature. Microplates for the assays were read spectrophotometrically at 450nm. Statistical analysis was done using fishers' t- test

Results:

A total of 162 stool specimens were analyzed for *C. difficile* from suspected cases of AAD. Of these 95 (58.64%) patients were males, whereas 67 (41.36%) were females. The median age of patients involved in the study was 41 days while the median duration of hospital stay was 7 days. The maximum number of *C. difficile* suspected cases were from gastrointestinal ward (59 cases, 36.4%), followed by internal medicine (51, 31.5%), oncology/ hematology ward (23, 14.19%), surgical ward (15, 9.26%) and other wards (14, 8.65%). A total of 96 percent of the analyzed group were on multiple antibiotics which included 56.12% on quinolones, 41% on cephalosporins, 15% on aminoglycosides and 13% on macrolides. 83.7% were on metronidazole or tinidazole and 11.11% on vancomycin, the commonly prescribed anti *C. difficile* medications.

Of the 162 stool samples investigated, a total of 7 samples were positive for *C. difficile* toxin. The prevalence of *C. difficile* associated diarrhea was found to be 4.32%. Highest number of *C. difficile* toxin positive cases were from stool samples of patients admitted in oncology/ hematology (3 cases) followed by internal medicine (2 cases). Clinical profile of patients with *C. difficile* associated diarrhea are summarized in Table 1.

Of the 7 positive cases, 4 (57.14%) were males while 3 (42.86%) were females. 4 patients experienced diarrhea within 2 weeks after the start of antibiotics. Median time of occurrence of symptoms was 7 days after admittance to hospital. All the patients were on multiple drugs and 71.4% per cent of the positive cases were on 3rd generation cephalosporins. *C. difficile* positive cases were treated with metronidazole or vancomycin. 3 of the 7 positive cases in our study were on chemotherapeutic agents and the association was found to be statistically significant ($p < 0.05$). All positive patients were on proton pump inhibitors but the association was not statistically significant.

Table 1.Clinical profile of patients with *C. difficile* infection in SKIMS, a tertiary care hospital

Age/sex	Diagnosis	Ward	Antibiotic types	Duration of hospital stay	Use of PPI	Recent surgery
36 /M	Type 1DM with sepsis	Gastroenterology	Cefoperazone-sulbactum, metronidazole	8d	Yes	Yes
68/M	CRF with pericardial effusion with enteritis	Internal medicine	Cefepime, levofloxacin, clindamycin, azithromycin	7d	Yes	No
45/M	Pyrexia of unknown origin	Internal medicine	Ceftriaxone, levofloxacin	28 d	Yes	No
32/F	Connective tissue disorder with Raynaud's disease	Surgery	Cefadroxil, amikacin	17d	Yes	Yes
50/F	Chronic diarrhea with carcinoma colon (resected)	Oncology/Hematology	Ofloxacin, tinidazole	14 d	Yes	No
75/F	Multiple myeloma	Oncology/ Hematology	Levofloxacin, vancomycin	7d	Yes	No
40/M	CML with GI bleed	Oncology/Hematology	Ceftriaxone, ofloxacin	20 d	Yes	No

d- days; PPI- proton pump inhibitors; Type 1DM- type 1 Diabetes mellitus; CRF- chronic renal failure; CML- chronic myeloid leukemia; GI bleed- gastrointestinal bleed.

Discussion:

Clostridium difficile has emerged as a major nosocomial pathogen and is a leading cause of antibiotic-associated diarrhea and pseudomembranous colitis. The diagnosis of *C. difficile* associated diarrhea is based on a combination of clinical criteria: Diarrhea (≥ 3 unformed stools per 24 h for ≥ 2 days), with no recognized cause and toxin A or B detected in stool (Shin et al., 2009). Toxins A and B mediate the pathogenesis of *C. difficile* infection (CDI), and toxin detection is an important part of diagnosis as described previously. Widespread unregulated antibiotic use, inappropriate prescribing and lacking surveillance measures in our setting indicate that CDI could be widespread. Studies on *C. difficile* performed in Asia demonstrate that CDI is a significant cause of nosocomial disease in Asian countries (Collins et al., 2013).

In our study, we used ELISA for diagnosing *C. difficile* infection. Standard laboratory methods for diagnosing these infections include stool culture and identification of bacterial isolate, fecal toxin detection and *C. difficile* antigen detection. The culture lacks specificity due to the possible fecal carriage of non-toxigenic isolates; therefore many laboratories rely on toxin detection rather than culture for diagnosis of *C. difficile* infection (Chaudhry et al., 2008). A cytotoxicity neutralization assay (CNA) is the reference method for toxin detection, but it is expensive and time-consuming and requires tissue culture facilities (Vaishnavi, 2010). Hence, we chose a commercial EIA to detect TcdA and/ or TcdB, with the benefits of rapid turnaround time and ease of use (Collins et al., 2013;Ticehurst et al., 2006; Musher et al., 2007; Peterson et al., 1988)

The prevalence of *C. difficile* associated diarrhea is global and the incidence varies from place to place. In our hospital, *C. difficile* was found to be responsible for 4.32% of cases of nosocomial diarrhea. A slightly higher prevalence of AAD (7-12%) in comparison to our study has been reported by some Indian authors (Chaudhry et al., 2008; Vaishnavi, 2010; Niyogi et al., 1991; Bhattacharya etal., 1991; Joshy et al2009) while other studies from India have reported a very high prevalence of 25-30% in hospitalized patients with diarrhea (Gupta and Yadav, 1985; Vaishnavi et al., 1999). In our study, a low prevalence of CDI can be attributed to a number of factors. First, poor recognition, awareness and surveillance of *C. difficile* associated infections. Despite the fact that patients undergoing surgery have a high risk of acquiring CDI, number of requested investigations was only 9.26%. Second, 80% of the patients were on anti *C. difficile* treatment by the time investigation for detection of toxin was requested. Third, the increasing practice of prescribing probiotics in patients who are on prolonged and broad spectrum antibiotic therapy in our hospital. Finally, lower prevalence in our study as compared to other studies could be because of lower sensitivity (60%–80%) of EIAs for toxins A and B compared with toxigenic stool culture as has been done in many studies (Lessa et al., 2012).

In our study, positive cases were mainly from patients in the hematology/ oncology, gastroenterology and general medicine wards. This points to the high risk areas for nosocomial spread of *C. difficile* isolates where broad spectrum antibiotics and immunosuppressive drugs are wide spread (Vaishnavi, 2010).

Multiple risk factors for antibiotic-associated diarrhea (AAD) have been described, including increasing age, length of hospital stay, and administration of broad-spectrum cephalosporins, broad-spectrum penicillins, and clindamycin (Asha et al., 2006). In the current study, the median age of positive cases was 45 years. The median duration of hospital stay was 7 days. Also, in the current study, positive cases were on combination and prolonged antibiotic therapy. The median time of occurrence of symptoms was 14 days after the start of treatment which is in accordance with other studies (Chaudhry et al., 2008; Thamlikitkul et al., 1996). Administration of antineoplastic drugs and proton pump inhibitors has also been implicated (Asha et al., 2006). In our study, the major risk factors of acquisition of CDI was administration of chemotherapeutic agents and the association was statistically significant. Use of PPIs as a risk factor could not be ascertained.

The current study draws attention to the role of *C. difficile* as a hospital-acquired pathogen in our hospital. For proper surveillance and future studies, clinicians need to be educated regarding this emerging pathogen as a cause of AAD for which high degree of clinical suspicion and sending requisition for testing of this pathogen before start of anti *C. difficile* drugs is necessary. Active and aggressive surveillance activity is required to reduce incidence and prevent its spread. Timely laboratory results of *C. difficile* testing can impact decisions regarding antibiotic therapy and infection control measures. Combined approach, involving effective control measures such as environmental hygiene, washing hands and isolating patients with CDI, as well as prudent use of antibiotics, is necessary for potentially significant improvement in patients' outcome.

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